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Donor genomics influence graft events: The effect of donor polymorphisms on acute rejection and chronic allograft nephropathy

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Donor genomics influence graft events: The effect of donor polymorphisms on acute rejection and chronic allograft nephropathy.

Background. Organs procured from deceased donors emanate from individuals with diverse genetic backgrounds. Donor organs, therefore, may vary in their response to injury and immune stimuli in a genetically determined manner. We assessed polymorphisms from 244 renal allograft donors to better understand the impact of donor polymorphisms on selected transplant outcomes.

Methods. Donor genomic DNA restriction fragment length polymorphisms were assayed for evidence of common cytokine [interleukin (IL)-2, IL-6, IL-10, tumor necrosis factor (TNF)-α, TGF-β, interferon (IFN)-γ] and chemokine (CCR2, CCR5) polymorphisms. Associations between donor polymorphisms and graft events were determined using chi-square, linear regression, and Kaplan-Meier analyses.

Results. Several genotypic polymorphisms demonstrated a modest association with acute rejection, including the transforming growth factor (TGF)-β T/C codon 10 ($P = 0.027$) and the CCR5 G/A 59029 ($P = 0.039$) genes by chi-square analysis. Notably, the presence of the T allele in the IFN-γ gene (+874) demonstrated a highly significant association with biopsy-proven chronic allograft nephropathy ($P < 0.008$). This association remained highly significant in a multiple linear regression model that incorporated biopsy-proven acute rejection as a covariate.

Conclusion. These data suggest that many of the donor polymorphisms studied in this analysis may influence a recipient's immune response to a renal allograft. However, their greatest impact may be demonstrated in long-term outcomes.

Key words: gene polymorphisms, donor populations, kidney transplantation.

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The genetic regulation of cytokine and chemokine gene expression is increasingly recognized as a significant variable affecting allograft outcome. Significant interindividual variations exist in many of the genes that regulate these molecules. This variation can be attributed, in part, to single nucleotide polymorphisms (SNPs) in the promoter region of a gene affecting the amount or rate of protein production, or the presence of variable number tandem repeats that can also alter the genetic regulation of a particular protein.

Investigations of genetic polymorphisms in solid organ transplantation have primarily dealt with recipient cytokine polymorphisms. Hutchinson et al studied tumor necrosis factor (TNF)-α and interleukin (IL)-10 gene polymorphisms in heart, liver, kidney, and simultaneous pancreas-kidney transplant recipients, and determined that a G/A polymorphism at position −308 in the recipients' TNF-α promoter was associated with an increased rate of rejection and an increased rate of steroid-resistant rejection in kidney transplant recipients [1, 2]. Poli et al demonstrated similar associations [3]. However, not all studies demonstrate such a clear relationship between genetic polymorphisms and outcomes. Marshall et al [4] were unable to delineate a significant contribution of a number of cytokine SNPs to the incidence or severity of acute rejection in a cohort of kidney transplant recipients.

These somewhat contradictory findings highlight the fact that the recipient genome has yet to yield a signature pattern of polymorphisms or genetic profile that defines transplant risk. This has spawned interest in examining the other half of the transplant puzzle, the donor organ. Many relevant immune responses, particularly effector responses, take place in the donor organ, a microenvironment that is often highly disparate from the recipient's own tissues. Our present understanding of organ-specific function now suggests that these tissues may play a

significant role in regulating immune responses, and their response to injury may vary considerably from one individual to another. Kidney tubular epithelial cells, for instance, express cytokines, chemokines, and many of their respective receptors in response to injury, many of which have both been implicated in acute rejection and shown to have polymorphic expression [5–9].

Specific cytokines that influence the complex molecular and cellular biology may play different roles in the context of transplant rejection and chronic allograft dysfunction. For instance, TNF-α may be very significant in the context of acute rejection; whereas transforming growth factor-beta (TGF-β) may play a more prominent role in chronic allograft dysfunction, although its potential involvement potentially in acute rejection is not to be discounted [10–12]. Chemokines also certainly play an important role in transplant rejection [13, 14]. Thus, any genomic examination of these transplant events must consider multiple genes in each family simultaneously.

Donor cytokine polymorphisms have recently been associated with delayed graft function [15], acute rejection [16], and reduced allograft survival [17]. We undertook this analysis to expand upon these initial studies by assessing the effects of combinations of polymorphisms on allograft events: acute rejection, chronic allograft nephropathy, and graft survival.

METHODS

Study type and DNA extraction

This was a case-cohort analysis, nonrandomized study examining donor samples. This study was approved by the appropriate University of Wisconsin and the National Institutes of Health (NIH) Institutional Review Boards. Donor splenocytes, obtained from the University of Wisconsin Histocompatibility laboratory or venous blood from living donors was collected in 5-mL EDTA-treated tubes prior to analysis. A subset of recipients of these organs ($N = 68$) also provided venous blood collected as above. This represented the set of recipients contacted who would provide informed consent at time of follow-up. DNA was isolated using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Donor and recipient samples were obtained from a single institution (UW). All donor samples were obtained from the 1997 to 1999 donor cohort at the institution.

Allograft function

Allograft biopsies were taken from recipients who received induction therapy utilizing depletion antibodies (rabbit antithymocyte globulin, Thymoglobulin®; Genzyme, Cambridge, MA, USA) or monoclonal CD25-specific antibodies (basiliximab or daclizumab) followed by maintenance immunosuppression with a calcineurin inhibitor (cyclosporine A or tacrolimus), mycophenolate mofetil, and/or prednisone. Target cyclosporine A levels were 150 to 200 ng/mL during the first year after transplantation. Target tacrolimus levels were 5 to 12 ng/mL during the first year after transplantation. Treatment was delivered with calcineurin inhibitors independent from this study and in keeping with standard of care. Biopsies were obtained from the renal cortex using a 16-gauge needle core biopsy device.

Individuals with increased serum creatinine (Scr) levels ≥0.3 mg/dL above baseline without another cause of graft dysfunction (e.g., urinary tract infection, obstructive uropathy, or individuals with proteinuria >1 g per day) underwent percutaneous graft biopsy. If rejection was present on the biopsy, individuals were treated originally with bolus corticosteroids (500 mg methylprednisolone) for three days then a corticosteroid taper. If the Scr or beta2-microglobulin level did not improve during that 72-hour period, rabbit antithymocyte globulin (Thymoglobulin® was added to the regimen at 1.5 mg/kg for 3 to 7 days). If chronic allograft nephropathy (CAN) was diagnosed by biopsy, the most common first step in management was a dose reduction in calcineurin inhibition. All biopsies were reviewed by pathologists using Banff criteria independent of the polymorphism analysis [11].

Characterization and detection of cytokine polymorphisms

All polymorphisms were determined by allele-specific polymerase chain reaction (PCR) with subsequent allele or genotype determination using methodologies from commercially available kits or restriction fragment length polymorphism (RFLP) analyses. Cytokine genotypes (IL-6, IL-10, TNF-α, TGF-β, and IFN-γ) were assessed using the One Lambda, Inc., Genotyping Kit (One Lambda, Inc., Canoga Park, CA, USA) [18]. Polymorphic differences in the IL-2 gene were determined following restriction enzyme digestion as described [18].

Characterization and detection of chemokine polymorphisms

CCR5-Δ32, CCR5-59029, and CCR2-V64I genotype. The CCR5-Δ32 genotype was determined by sizing PCR amplicons that include the entire region of the deletion [19]. PCR was performed using 50 ng of genomic DNA in a reaction mix that contained 1× Taq polymerase buffer (Applied Biosystems, Inc., Foster City, CA, USA), 0.2 mmol/L dNTP, 150 ng each primer and 0.2 U of Taq Polymerase (Applied Biosystems, Inc.). Thermocycling (PTC 100; MJ Research, Watertown, MA, USA) consisted of initial denaturation at 94°C for 4 minutes followed by 35 cycles of 94°C for 30 seconds, 52°C for 45 seconds, and 72°C for 1 minute, and final extension at 72°C for 7 minutes. Amplicons were visualized under UV light on 2% agarose gel containing ethidium bromide. The sense primer was 5′-TGTTTGCGTCTCTCCCAG-3′,

and antisense was 5′-CACAGCCCTGTGCCTCTT-3′. These primers yield a 233 and 201 bp product for the wild-type and deletion amplicons, respectively.

CCR5–59029 and CCR2-V64I genomic variants were detected using PCR conditions identical to those for CCR5-Δ32, except for an annealing temperature of 65°C. Sense, 5′-CCCGTGAGCCCATAGTTAAAACTC-3′, and antisense, 5′-TCACAGGGCTTTTCAACAGTAAG G-3′, primers were used for PCR for CCR5-59029 genomic variants. This reaction yielded a 268-bp amplicon. The PCR product was digested with 10 units of *Bsp*1286I (New England BioLabs, Beverly, MA, USA). Amplicons from individuals homozygous for 52909-G appear as a single ∼130-bp band after *Bsp*1286I digestion. Individuals homozygous for 59029-A have a 258-bp product, while heterozygotes display both bands.

The sense primer for the CCR2-V64I genotype was 5′-TTGGTTTTGTGGGCAACA TGATGG-3′, and the antisense primer was 5′-CATTGCATTCCCAAAGACC CACTC-3′. Amplification of the PCR product resulted in a 173-bp product. An A at nucleotide position 190 encodes isoleucine at position 64 and yields restriction fragments of 149 and 24 bp after *Bsa*BI digestion. In contrast, the 173-bp amplicon remains uncut if a G encoding a valine is present.

Statistical analysis

Donor samples were genotyped. Graft events, biopsy-proven acute cellular rejection; biopsy-proven CAN, and graft loss defined as return to dialysis or retransplantation, were obtained from the transplant center database. If a donor organ was associated with any of these events, it was tabulated as that for the donor, regardless of the outcome of the other kidney. Each polymorphism was tested for Hardy-Weinberg equilibrium via a two degree-of-freedom chi-square goodness of fit. The transplant outcome measures were cross-tabulated against the phenotype derived from each polymorphic genotype. Transplant outcome measures (e.g., rejection), were then tested via Fisher exact test with Bonferroni-Holm correction. Differences among rejectors versus nonrejectors when comparing increased versus low phenotypes were determined following 2 × 2 contingency and chi-square analysis (JMP, SAS Institute, Cary, NC, USA). A power calculation determined that a donor population of 200 individuals would be necessary to determine a significant difference in the effect of homozygosity of a specific allele based on its distribution in the population led to a significant association (>3-fold increase in incidence) with acute rejection, based on a historic acute rejection rate of 38% at our institution versus heterozygosity or homozygosity of the other allele ($\alpha = 0.05$). We also performed Kaplan-Meier estimates of time to acute rejection. Comparisons between groups bearing individual polymorphisms were evaluated using the log-rank test. P values < 0.05 (two-sided) were considered significant.

Next, we developed a multiple linear regression model examining acute rejection or CAN as the dependent variable. We correlated any event with the donor as a single categorization, based on the intent of the study. The independent variables in each model were donor age, cold ischemia time, recipient age, delayed graft function, recipient age, gender, degree of human leukocyte antigen (HLA) mismatch, depletion versus monoclonal antibody induction therapy, and acute rejection for the CAN model. Predicted phenotypes defined by the aforementioned polymorphisms and genotype analyses were then analyzed for their potential independent effects on each event.

To investigate the interaction between genetic polymorphisms, and to potentially strengthen the power of the analysis, we reduced the predicted phenotypes associated with the graft events in the aforementioned analyses to one genetic variable [20] and reassessed the impact of donor genomics in the model. Thus, any genotype associated with a readily described phenotype that had an independent association with acute rejection (e.g., TGF-β, CCR5 59029A, or CAN) were grouped into the composite variable.

Table 1. Donor and recipient demographics

Donors *N*	244
Average age *years* ± SD	39.2 ± 15.4
Gender (F/M)	103 (42%)/141 (58%)
Deceased donor/living donor	232 (95%)/12 (5%)
Cold ischemia time *hours*	16.2 ± 11.5
Average no. HLA mismatches	2.3 ± 2.1
Donors after cardiac death/ extended criteria donors	39 (16%)/29 (12%)
Recipients *N*	379
Average age *years* ± SD	49.3 ± 16.7
Gender (F/M)	148 (39%)/231 (61%)
Caucasian/African-American/other	326 (86%)/45 (12%)/8 (2%)

The vast majority of the donor population was Caucasian (>90%). Twenty-one percent of the deceased donors would be classified as extended criteria donors.

RESULTS

Demographics of the study populations

Donor demographics are summarized in Table 1. The vast majority of the 244 donors were deceased donors (95%) and Caucasian (91%). When possible, donor samples were genotyped for every polymorphism examined. However, in some instances, sample volume or technical complications related to repeated assays may have limited testing for every allelic difference examined. The overall number of donors genotyped for each cytokine and chemokine allelic polymorphisms examined were: TNF-α ($N = 240$), TGF-β ($N = 234$), IFN-γ ($N = 239$), IL-2 ($N = 244$); IL-6 ($N = 242$), IL-10 ($N = 233$), CCR5 ($N = 239$), and CCR2 ($N = 239$).

Table 2. Cytokine genotype polymorphism distribution in allograft donor population

Gene	Position	Genotype	Phenotype	Non-rejection	Acute rejection	Chi-square
IL-2	−330	G/G	High	6	3	NS, $P = 0.25$
		T/G	Low	48	49	
		T/T		82	56	
IL-6	+174	G/G	High	51	34	NS, $P = 0.22$
		G/C		64	27	
		C/C	Low	21	45	
IL-10	−1082	GCC/GCC	High	23	15	NS, $P = 0.21$
		GCC/ACC		36	35	
	−819	GCC/ATA		29	23	
		ACC/ACC	Low	4	6	
	−592	ACC/ATA		28	17	
		ATA/ATA		5	12	
TNF-α	−308	A/A	High	34	39	NS, $P = 0.25$
		G/A		74	57	
		G/G	Low	25	11	
TGF-β	Codon 10	T/T G/G	High	52	31	$P = 0.027$
		T/C G/G		35	47	
		T/C G/C		16	14	
	Codon 25	C/C G/G	Low	16	7	
		C/C G/C		4	6	
		C/C C/C		0	2	
		T/T C/C		3	1	
IFN-γ	+875	T/T	High	25	11	NS, $P = 0.074$
		T/A		73	57	
		A/A	Low	34	39	

Cytokine gene polymorphisms and predicted phenotype distribution based on acute rejection diagnosed in the donor graft. All analyses were performed as described in **Methods**.

The donor cohort was separated into groups based on recipient transplant outcomes. Initial chi-square analyses were performed separating the groups into nonrejecting donors ($N = 136$) and donors with recipient biopsy-proven acute rejection ($N = 108$). Donor and recipient gender, age, and race/ethnicity were recorded, and individual donors were genotyped as high or low gene protein producers as previously established [1, 2, 12]. Observed frequencies were not significantly different from the expected frequencies under Hardy-Weinberg equilibrium conditions.

Adequate follow-up and outcome data were available for 379 individuals who received kidneys from these donors at the University of Wisconsin. Individuals who received combined organ transplants (e.g., kidney and pancreas transplantation) were excluded from the analysis. The majority of these individuals were male and Caucasian (Table 1).

Acute rejection

As described in **Methods**, we examined for potential associations between donor polymorphisms and acute rejection. We observed associations between select genes and acute cellular rejection. When the donor TGF-β alleles were separated into conglomerate genotypes, those haplotypes linked with increased protein production were significantly associated with grafts that manifested acute cellular rejection ($P = 0.027$).

We also investigated select chemokines polymorphisms. In the context of this, one analysis examined functional versus impaired CCR5, based on the predicted phenotype of the genotype as opposed to simply high or low protein production (Table 3). The CCR5 59029A allele was more prevalent in donor kidneys that experienced acute cellular rejection ($P = 0.029$) (Table 3). Essentially the same statistical results were obtained when only deceased donors were assessed. The donor TGF-β haplotypes associated with increased protein production were associated with grafts that manifested acute cellular rejection ($P = 0.023$). The CCR5 59029A allele also was linked with donor kidneys that experienced acute rejection ($P = 0.027$). No relationship was evident between other allelic or donor polymorphisms tested and acute cellular rejection.

The high-producing IFN-γ T allele at +875, when expressed in donors, was also associated with a trend toward acute rejection.

Chronic allograft nephropathy

The CCR5 59029A allele also demonstrated an association with biopsy-proven CAN ($P < 0.04$). However, more striking was the highly significant association between the presence of the T allele associated with increased production of IFN-γ and CAN ($P = 0.007$) (Table 4).

Again, when only deceased donor samples were examined, the same results held (CCR5 59029A: $P = 0.03$; IFN-γ: $P = 0.002$). We observed no other significant associations between alleles, polymorphisms, or predicted phenotype and outcomes, save for a moderate effect of

Table 3. Chemokine genotype polymorphism distribution in allograft donor population

Gene	Position	Genotype	Phenotype	Non-rejection	Acute rejection	Chi-square
CCR5		+/+	Functional	102	81	NS, 0.4
		+/−		32	22	
		−/−	Impaired	1	1	
CCR5	59029	G/G	High	30	18	$P = 0.029$
		G/A		75	56	
		A/A	Low	30	30	
CCR2	+190	+/+	High	109	82	NS, 0.94
		+/64I		22	18	
		64I/64I	Low	4	4	

Chemokine gene polymorphisms and predicted phenotype distribution based on acute rejection diagnosed in the donor graft. All analyses were performed as described in **Methods** using chi-square analyses.

Table 4. Donor allelic and genotype polymorphisms and biopsy-proven CAN

Polymorphism	*P* value (Fisher exact)
IL-2 –330 G	NS, $P = 0.16$
IL-6 –174G	NS, $P = 0.34$
IL-10 GCC	NS, $P = 0.91$
TNF-α –308A	NS, $P = 0.84$
TGF-β codon 10 T or codon 25 C	NS, $P \geq 0.78$ for each
IFN-γ +875 T	$P = 0.0076$
CCR5 (+/+)	NS, $P = 0.166$
CCR5 59029-A	$P = 0.037$
CCR2 +190 (+/+)	NS, $P = 0.79$

Allelic and genotype polymorphisms in association with biopsy-proven chronic allograft nephropathy in the donor graft. All analyses were performed as described in **Methods** using Fisher exact test with Bonferroni-Holm correction.

the increased TNF-α production phenotype and graft failure ($P = 0.04$).

Linear regression analysis

The linear regression models attempted to take advantage of variables known to affect acute rejection and CAN and determine if donor genomics affect those events (Table 5). The donor age range was 6 to 68 years. The delayed graft function rate (per donor) was 31% ($N =$ 75 donors). Fifty-three percent ($N = 129$) of the donor-related kidney transplants were treated with monoclonal antibody induction. In the acute rejection model, donor age [relative risk (RR) 1.14; $P = 0.01$); delayed graft function (RR 2.1; $P = 0.02$); and the degree of HLA mismatch (RR 1.78; $P = 0.003$) all were significantly associated with acute rejection. The increased TGF-β production predicted phenotype demonstrated a modest effect (RR 1.28; $P = 0.019$). The donor CCR5 (increased receptor expression), low-producing IFN-γ, and IL-2 phenotypes did not manifest significant independent effects. The combination phenotype variable demonstrated a moderately significant effect on acute rejection (RR 1.22; $P = 0.033$).

In the CAN model, delayed graft function had a modest though not significant effect on CAN (RR 1.35; NS, $P = 0.072$). Donor age (RR 2.44; $P = 0.001$) and acute rejection (RR 1.47; $P = 0.01$) were significantly associated with CAN. The donor CCR5 increased receptor expression phenotype did not appear to have an independent effect. However, the increased IFN-γ production phenotype displayed a highly significant independent effect on the development of CAN (RR 1.73; $P = 0.001$). We then re-examined the model with the combination phenotype variable for the phenotypes associated with adverse outcomes/function in CAN. This component variable also demonstrated a significant independent effect on the evolution of CAN (RR 1.47; $P = 0.008$).

Kaplan-Meier analyses

We also analyzed the alleles and polymorphisms associated with the rate of biopsy-proven acute rejection by Kaplan-Meier methodology. Interestingly, there was a trend in time to acute rejection in donors that manifested the codon 10 TGF-β haplotypes associated with high levels of protein production (NS, $P = 0.077$). There were not any significant differences in time to acute rejection among the other individual alleles tested.

We then examined if the presence of the codon 10 TGF-β haplotype in both donor and recipient led to an increased rate of rejection. We compared this group ($N = 11$) to the other donor/recipient combinations in aggregate (+ donor/-recipient; —donor/+ recipient; and— donor/− recipient) in terms of rate of acute rejection. There was again a trend toward a more rapid rate of acute rejection in the + donor/+ recipient group ($P = 0.062$) (Fig. 1). A similar analysis examining time to a composite endpoint of biopsy-proven CAN or allograft failure in + donor/+ recipient increased IFN-γ phenotype ($N = 24$) versus the other categories in aggregate demonstrated a significant difference ($P = 0.01$; Fig. 2). There was no significant difference in the rate of reaching this composite outcome point when comparing the CCR5 phenotype combinations.

DISCUSSION

The recipient has been the focus of most of the transplantation genomics studies to date. However, the consequences of donor-recipient interactions occur as a result of the net effects of both donor- and recipient-derived

Table 5. Linear regression model: Impact of donor genomic variables on acute rejection and chronic allograft nephropathy

Parameter	Acute rejection		Chronic allograft nephropathy	
	Relative risk (95% CI)	*P* value	Relative risk (95% CI)	*P* value
Donor age	1.14 (1.06; 1.31)	$P = 0.01$	2.44 (1.31; 3.34)	$P = 0.001$
DGF	2.1 (1.3; 3.03)	$P = 0.02$	1.35 (0.97; 1.79)	NS, $P = 0.074$
Recipient age	1.05 (0.87; 1.95)	NS	0.97 (0.89; 1.07)	NS
CIT	1.57 (0.88; 1.98)	NS	1.92 (0.93; 3.78)	NS
Gender (M vs. F)	0.94 (0.84; 1.11)	NS	1.34 (0.65; 2.31)	NS
Depletion vs. monoclonal antibody	0.81 (0.67; 1.05)	NS	0.89 (0.66; 2.78)	NS
Degree HLA mismatch	1.78 (1.56; 1.97)	$P = 0.003$	1.36 (0.92; 1.91)	NS
↑ TGF-β phenotype	1.28 (1.07; 1.49)	$P = 0.019$	–	–
CCR5 59029A genotype (↑ receptor expression)	1.78 (0.92; 3.15)	NS	1.14 (0.94; 1.26)	NS
Low-producing IFN-γ	1.63 (0.91; 3.43)	NS	–	–
↑ IFN-γ phenotype	–	–	1.73 (1.60; 1.88)	$P = 0.001$
↑ IL-2 phenotype	1.45 (0.76; 2.36)	NS	–	–
Composite phenotypic variable	1.22 (1.02; 1.48)	$P = 0.033$	1.47 (1.33; 1.61)	$P = 0.008$
Acute rejection	–	–	1.47 (1.26; 1.69)	$P = 0.01$

Results of linear regression examining potential impact of predicted phenotype of alleles and polymorphisms shown to be significant in the chi-square and Fisher exact analyses. Analyses for each model were performed as described in **Methods**. Genomic variables that were not significant for acute rejection or chronic allograft nephropathy in the aforementioned analyses were not included in the model for that condition (e.g., the low-producing IFN-γ phenotype was not tested in the chronic allograft nephropathy model). A composite predicted phenotypic variable was incorporated into the model to increase its power. Acute rejection was a covariate in the chronic allograft nephropathy model.

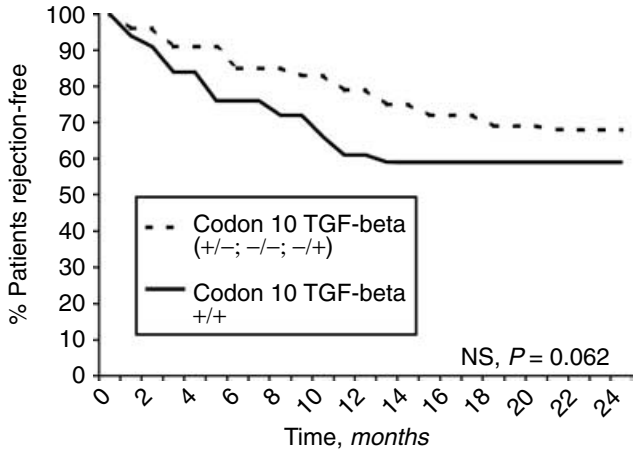

Fig. 1. Kaplan-Meier analysis of time to biopsy-proven acute rejection. Donor/recipient pairs positive for the increased TGF-β1 producing haplotype (codon 10) had a trend toward a more rapid rate to time to acute rejection than donor +/recipient −; donor–/recipient +; and donor–/recipient—pairs (NS, $P = 0.062$).

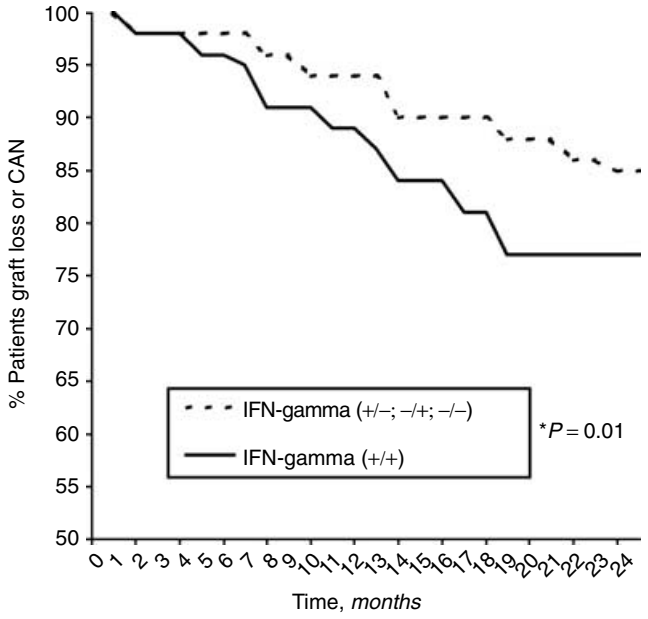

Fig. 2. Kaplan-Meier analysis of time to composite endpoint of biopsy-proven chronic allograft nephropathy (CAN) or graft loss. Donor/recipient pairs positive for the IFN-γ increased production phenotypes (based on presence of T allele) had a significantly greater rate of reaching the composite end point than donor +/recipient −; donor–/recipient +; and donor–/recipient pairs ($P = 0.01$).

factors, and donor tissues can vary both in their ability to stimulate immunity and in their ability to heal after injury. For example, early rejection episodes can be affected by donor age and ethnicity [17, 18]. Other forms of injury (e.g., brain death and procurement), alter intragraft cytokine and adhesion molecule production, thereby setting the stage for additional allograft damage [21–23]. Thus, an accurate assessment of donor-specific variables has significant implications, especially given the increased interest in extended-criteria donors [24]. This study suggests that select donor genes could be incorporated as donor-specific variables that influence acute rejection and CAN, emphasizing the importance of the donor genetic makeup beyond traditional HLA matching.

The most striking finding in our study was that the allele associated with increased IFN-γ production in donors significantly increased the likelihood of CAN. There is no doubt that increased recipient IFN-γ expression is associated with chronic allograft dysfunction [25]. However, recently the focus on this cytokine has shifted to its effect within donors, *per se* [26]. Halloran et al suggested an essential role for IFN-γ in preventing necrosis early during

a rejection process, but in so doing, it potentiated chronic allograft dysfunction [26]. The latter effect certainly could explain, in part, the data presented herein. Additionally, for secreted proteins, the source of the molecule may be less relevant than the absolute amount present in the intragraft milieu. In this regard, both donor and recipient contributions may be equally relevant.

The intriguing aspect about such observations is that they suggest the donor organ may have a role in its own fate. There are several mechanisms through which donor grafts could credibly influence graft outcome. High expression polymorphisms could increase intragraft concentrations of cytokines for instance, luring increased numbers of recipient cells to the allograft to initiate or augment an immune response. Cytokines are not allospecific, and donor-derived proinflammatory cytokines can influence recipient-derived cells. Wong et al [27] recently described the integral role of exogenous TGF-β1 in perpetuating tubulitis and in enhancing CD8+ T cell proliferation. Their studies suggest a mechanism by which intragraft TGF-β1 could have a direct effect in the development of acute rejection, akin to that described above.

We also determined TGF-β1 codon 10 "status" and IFN-γ "status" in a manner akin to that which we do clinically to assess cytomegalovirus status. Individual donor +/recipient + pairs demonstrated either a trend toward increased rejection rates in the case of TGF-β1 codon 10, or a significantly increased rate of graft loss or CAN in the case of IFN-γ increased production phenotype. Though an indirect approach, these results further suggest that donor genomics bear consideration in terms of donor selection as they could influence not just the graft but potentially alter donor-recipient interactions in a way that affects graft outcomes.

Another possibility is that donor-derived passenger mononuclear cells might express more receptor proteins. These cells, coursing through the graft, would bind ligands, initiating and exacerbating intragraft inflammation after transplantation. In this regard, the association between donor homozygosity for the CCR5 59029-A allele and acute rejection is of interest. This particular allele has been linked with a reduction in acute allograft rejection in kidney transplant recipients [19], but its presence in donors was associated with an increased risk for rejection. CCR5 is a member of the chemokine receptor superfamily. A wide array of immune responses during renal allotransplantation are triggered by chemokines and chemokine receptors [28–30]. CCR5, a member of the chemokine receptor superfamily, and its ligands (MIP-1α, MIP-1β, and RANTES) are elevated in both subclinical and clinical acute rejection [26]. CCR5 is expressed predominantly on dendritic cells, memory cells, and monocytes, and thus, its presence on donor-derived passenger mononuclear cells could be invoked to explain the findings herein. It is also intriguing that CCR5 has been identified on vascular smooth muscle cells [31]. This raises an alternative hypothesis, that endothelial-vascular smooth muscle interactions in the allograft could be a site for important CCR5 effects. Additional studies will be necessary to explicitly examine the role of CCR5 on donor cells in the setting of transplantation.

The associations between recipient polymorphisms and transplant outcomes (e.g., acute rejection or graft survival) have been examined in several studies [19, 32–36]. Recent work also has demonstrated interest in the potential association between donor polymorphisms and transplant outcomes [16, 32, 36, 37]. Marshall et al identified an association between a polymorphism at position –174 in the donor IL-6 gene and acute rejection in a single-center study [16]. St. Peter et al [15] also noted an association between polymorphisms in one of three classes of glutathione-S-transferase in donor grafts and delayed graft function. Finally, in another analysis, the donor G protein β3 subunit 825T genotype was linked with reduced allograft survival [17]. Our study represents another step toward understanding transplantation genomics by examining multiple polymorphisms and events simultaneously. The polygenic approach that we undertook is by no means complete, however, and may have excluded potentially significant genes involved in transplantation.

CONCLUSION

Our data suggest that donor genetic heterogeneity has a role in the assessment of donor risk factors and allograft outcomes. Yet this conclusion is accompanied by certain caveats. This particular donor population was skewed toward Caucasians. Racial and ethnic groups demonstrate alterations in expressions of certain polymorphisms [26]. Therefore, it is imperative to note that multicenter prospective studies that compile donors from varying racial and ethnic backgrounds will be necessary to establish the validity of donor polymorphisms as important transplant variables. Second, the data herein apply only to kidney transplantation. It is possible that certain donor polymorphisms that have a negative impact in kidneys could be beneficial in other organs. Finally, the rate of biopsy-proven rejection in this donor population is high. We purposely maintained the categorization of rejection in relationship with the donor, given the intent of the study. Even in shifting focus to the recipient number, this rate of biopsy-proven rejection remains high. This could be possibly due to oversampling in this population as there are more than 600 kidney transplant biopsies, on average, per year at the University of Wisconsin. Moreover, a confounding factor embedded in this problem is the potential inability to adequately distinguish nonspecific inflammation from rejection with simply histologic assessment of the tissue. Such testing, while

the subject of a number of reports and energetic experimentation, is not yet perceived as a clinical standard. Nonetheless, the study schema that we utilized with multiple genes and diverse outcome analyses sets the stage for expanded prospective analyses of genomics in all forms of transplantation. Moreover, it recognizes the simple but significant fact that the allograft itself is an important mediator and potential contributor to injury within its environment.

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